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Packaging and Cold Shipping of Human Islets V.2

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Integrated Islet Distribution Program[1]

[1]Integrated Islet Distribution Program, City of Hope, Duarte, CA

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Integrated Islet Distribution Program
Integrated Islet Distribution Program, City of Hope

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Abstract

This SOP defines a standardized method for packaging and cold shipping of research quality islets to approved investigators of human isolated islet preparations, for use in the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) sponsored research in the Integrated Islet Distribution Program (IIDP). This protocol is written to assist the participating islet isolation centers and investigators who are part of this program.

Note

Integrated Islet Distribution Program (IIDP) (RRID:SCR_014387)

Note: This SOP was developed based on the Prodo Labs, Inc. shipping protocol and results from preliminary studies conducted by the IIDP and commissioned by the original IIDP Project Officer, and External Evaluation Committee (EEC).

Guidelines

Responsibilities:

- It is the responsibility of the IIDP CC to both follow and ensure adherence to the procedures outlined in this SOP. In order to accomplish this, the IIDP CC will interact with the relevant personnel from each of the participating centers.

- It is the responsibility of each IIDP center to follow the procedures listed in this SOP and to work to the best of their ability to follow all requirements.

Definitions:

- ***Integrated Islet Distribution Program (IIDP) (RRID:SCR_014387):*** The IIDP is a program commissioned and funded by the NIDDK to provide quality human islets to the diabetes research community to advance scientific discoveries and translational medicine. The IIDP consists of the NIDDK Project Scientist and Program Official, the External Evaluation Committee and the CC at City of Hope (COH). The IIDP CC integrates an interactive group of academic laboratories including the subcontracted IIDP centers.

- ***IIDP Coordinating Center (CC):*** Joyce Niland, Ph.D., IIDP Principal Investigator leads CC staff to coordinate the activities of the IIDP and assists the participating centers and investigators in the distribution of human islets.

- ***Islet Equivalent (IEQ):*** An IEQ is defined as an islet with a diameter of 150 microns. The number of IEQ in each size class is calculated by multiplying the number of islets by a conversion factor for each micron size.

- ***Approved Investigators:*** Researchers who have requested islets from the IIDP for basic science studies and whose research protocols have been reviewed and approved by the IIDP.

- ***Islet Allocation System (IA):*** This is the online system administered by the IIDP to allow fair distribution of basic science islets to approved investigators. This interactive System is used by the IIDP Centers and the Approved Investigators facilitates and tracks the distribution of human islets.

Materials

MATERIALS

- Dynamic Diagnostics TransPak™ Pre-filled 10% Neutral Buffered Formalin (NBF) **Fisher Scientific Catalog #**22-899-402
- Corning™ Ciprofloxacin Hydrochloride **Fisher Scientific Catalog #**MT61277RG (Corning™ 61277RG)
- Human AB Serum (ABS) HI **Gemini Bio-Products Catalog #**100-512; Heat Inactivated
- PIM(G)® (5 mL Glutamine/Glutathione) **Prodo Laboratories, Inc Catalog #**PIM(G)®
- PIM(T)® **Prodo Laboratories, Inc Catalog #** PIM(T)®

Equipment

Phase 22 PCM Flex Pack

NAME

Phase 22 cold packs to maintain temperature at 22C

TYPE

TCP Reliable Manufacturing

BRAND

FMIS76000

SKU

https://www.cryopak.com/packaging-and-refrigerants/phase-change-materials/phase-22/

LINK

SPECIFICATIONS

•Most used for applications that need to maintain a controlled room temperature
•Phase change temperature is 22°C
•Provides thermal protection when shipping products between 15-30°C

Equipment

Thermo Scientific™ Nalgene™ Square PETG Media Bottles with Closure

NAME

30 mL PETG Media Bottles with Closure

TYPE

Thermo Scientific™ Nalgene™

BRAND

03-311-1V

SKU

https://www.fishersci.com/shop/products/nalgene-square-petg-media-bottles-closure/033111V#?keyword=true

LINK

30 mL PETG Media Bottles

SPECIFICATIONS

Equipment

PETG Media Bottles with Closure

NAME

60 mL PETG Media Bottles with Closure

TYPE

Thermo Scientific™ Nalgene™ Square PETG Media Bott

BRAND

03-311-1W

SKU

https://www.fishersci.com/shop/products/nalgene-square-petg-media-bottles-closure/033111W#?keyword=true

LINK

PETG Media Bottles

SPECIFICATIONS

Equipment

Media Bottles with Closure

NAME

125 mL PETG Media Bottles with Closure

TYPE

Thermo Scientific™ Nalgene™

BRAND

03-312-1

SKU

https://www.fishersci.com/shop/products/nalgene-square-petg-media-bottles-closure/033121#?keyword=true

LINK

PETG Media Bottles

SPECIFICATIONS

Equipment

Durasorb™ Underpads

NAME

Underpads

TYPE

Covidien

BRAND

22-031-340

SKU

https://www.fishersci.com/shop/products/covidien-durasorb-underpads/22031340#?keyword=22+031+340

LINK

Wings™ Fluff Underpad 17 × 24 in.

SPECIFICATIONS

Equipment

Reclosable Zip Bags

NAME

Clear Reclosable Zip Bags

TYPE

RD Plastics

BRAND

19-130-6043

SKU

https://www.fishersci.com/shop/products/rd-plastics-clear-reclosable-zip-bags-6/191306043?keyword=true

LINK

PE (Polyethylene) Reclosable bag (8 × 10 in.)

SPECIFICATIONS

Equipment

Uline Corrugated Boxes

NAME

Corrugated Boxes (Inner Shipping Box)

TYPE

Uline

BRAND

S-4344

SKU

https://www.uline.com/Product/Detail/S-4344/Corrugated-Boxes-200-Test/7-x-5-x-5-Corrugated-Boxes?keywords=S-4344+7+x+5+x+5%22+Corrugated+Boxes

LINK

7 × 5 × 5" Corrugated Boxes

SPECIFICATIONS

Equipment

Insulated Foam Shipping Kit

NAME

Insulated Foam Shipping Kit

TYPE

Uline

BRAND

S-7359

SKU

https://www.uline.com/Product/Detail/S-7359/Insulated-Shippers-and-Supplies/Insulated-Foam-Shipping-Kit-8-x-6-x-9?keywords=S-7359

LINK

Insulated Foam Shipping Kit - 8 × 6 × 9"

SPECIFICATIONS

Equipment

Uline Cold Pack

NAME

Cold Pack

TYPE

Uline

BRAND

S-7361

SKU

https://www.uline.com/Product/Detail/S-7361/Insulated-Shippers-and-Supplies/Cold-Packs-8-oz?keywords=S-7361

LINK

Reusable, leakproof refrigerant gel stays colder than ice. Thick 4 mil poly. 6 × 4 × 3/4"

SPECIFICATIONS

Equipment

Uline Industrial Tape	NAME
Uline Industrial Tape - 2 Mil, 2" x 110 yds, Clear	TYPE
Uline	BRAND
S-423	SKU
https://www.uline.com/Product/Detail/S-423/Carton-Sealing-Tape/Uline-Industrial-Tape-2-Mil-2-x-110-yds-Clear?keywords=S-423	LINK
2 Mil, 2" x 110 yds, Clear. DURABLE: Industrial Tape stands up to heat, humidity and cold.	SPECIFICATIONS

Equipment

Custom Cold Chain Complete Card	NAME
Temperature Monitoring system	TYPE
Tip TEMPerature Products	BRAND
WMSSEN044;	SKU
https://www.tiptemp.com/	LINK
Custom Cold Chain Complete Card	SPECIFICATIONS

Equipment

Tip TEMPerature Products

NAME

WarmMark Ascending Temperature indicator

TYPE

WarmMark Ascending Temperature indicator

BRAND

WMSSEN009

SKU

https://www.tiptemp.com/Products/Rising-Time-Temperature-Indicating-Labels/WMSSEN009-WarmMark-with-Trigger-Temperature-37C-99F.html

LINK

Maximum exposure above 37°C/99°F

SPECIFICATIONS

Equipment

ColdMark Descending Temperature Indicator

NAME

Descending Temperature Indicator

TYPE

Tip TEMPerature Products

BRAND

CLMSEN004

SKU

https://www.tiptemp.com/Products/Falling-Temperature-Indicating-Labels/CLMSEN004-Temperature-Label-ColdMark-2C-36F.html

LINK

Falling Temp Monitor Changes Color Permanently; 2°C/36°F

SPECIFICATIONS

Equipment

Laser Labels	NAME
Uline Laser Labels - White, 8 ½ x 11"	TYPE
Uline	BRAND
S-5045	SKU
https://www.uline.com/Product/Detail/S-5045/Laser-Labels/Uline-Laser-Labels-White-8-1-2-x-11?keywords=S-5045+Uline+Laser+Labels+-+White%2c+8+1%2f2+x+11%22	LINK
Box of 100 Single Labels, White, 8 ½ x 11"	SPECIFICATIONS

Troubleshooting

Safety warnings

10% Neutral Buffered Formalin: SDS_DYNDIA_FORMALIN_PREFILL_...

- Hazard Statement(s): Harmful if inhaled. Causes skin irritation. Causes serious eye damage. May cause allergy or asthma symptoms or breathing difficulties if inhaled. May cause an allergic skin reaction. May cause genetic defects. May cause cancer. May cause damage to organs (lungs, nose). May cause damage to organs (lungs, nose) through prolonged or repeated exposure.
- Precautionary Statement(s): Prevention: Avoid breathing dust, vapors. Use only outdoors or in a well-ventilated area. Wash body thoroughly after handling. Wear protective gloves. Wear eye protection, face protection. Wear NIOSH approved respiratory protection. Contaminated work clothing must not be allowed out of the workplace. Obtain special instructions before use. Do not handle until all safety precautions have been read and understood. Wear protective gloves, protective clothing, eye protection and face protection. Do not breathe dust, vapors. Do not eat, drink or smoke when using this product.

Ciprofloxacin Hydrochloride SDS-61-277-AGHS-EN-2014Jul22....

Precautionary statements:

- P280 - Wear protective gloves and eye/face protection
- P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- P337 + P313 - If eye irritation persists: Get medical advice/attention.
- P273 - Avoid release to the environment.

GemCell™ U.S. Origin Human Serum AB sds_100-512_gemcell_hsab.pdf

GemCell™ human serum AB is collected from healthy male donors of the AB serotype at FDA-licensed facilities in the United States.

Hazardous Components:

- Biohazard contains human source material. Handle as though capable of transmitting infectious agents.
- Toxicity: Not Established.
- Target Organs/Systems: Product could possibly irritate the skin, eyes and respiratory system. Do not ingest this product.

Before start

References:

- Tip Temperature Products website: http://www.tiptemp.com and product inserts
- Prodo Labs, Inc. Protocols and Website: http://www.prodolabs.com

Prodo Media-Preparation-and-Use...

Citation

David W. Scharp, Jayagowri Arulmoli, Kelly Morgan, Hannah Sunshine, Ergeng Hao (2019)
. Advances in Human Islet Processing: Manufacturing Steps to Achieve Predictable Islet Outcomes from Research Pancreases.
OBM Transplantation.

10.21926/obm.transplant.1901052 LINK

Citation

Ricordi C, Gray DWR, Hering BJ, Kaufman DB, Warnek GL, Kneteman N, et al. (1990)
. Islet isolation assessment in man and large animals.. Acta Diabetol.lat. 1990; 27: 185-195.

http://www.ncbi.nlm.nih.gov/pubmed/2075782 LINK

Materials

1 The IIDP will provide each center with the supplies necessary for shipments as listed in the materials section. Standard lab supplies will be provided by the IIDP Centers as listed below.

1.1 **Supplies provided by the IIDP Centers:**

- Islets for distribution
- Routine lab supplies for transferring islets
- Completed FedEx Shipping Labels
- Preprinted Fresh Tissue Box Labels
- Islet Allocation Tissue Shipment Forms for appropriate recipients
- Markers for labeling vessels

1.2 **Supplies provided by the IIDP:**

The majority of supplies should be stored in appropriate dry, temperature-controlled environments (room temperature 16°-28°C).

- The Prodo Labs PIM(T)® should be stored, in the dark, between 2° and 8°C upon receipt but is stable at room temperature.
- The Gemini AB serum and the PIM(G)® vials should be stored at -5° to -20°C in the dark.
- The Ciprofloxacin can be stored on the shelf but filter sterilized suspension aliquots should be stored at -5° to -20°C. Aliquots:

Pre-Preparation of Ciprofloxacin Powder for Addition to Media

- Remove 0.5 gm (500mg) of ciprofloxacin hydrochloride from the bottle and QS to 50mL with distilled water. This will give a stock concentration of 10mg/ml.
- Mix with a stir bar and stirring plate until totally dissolved.
- Filter sterilize the solution using a 0.2µM filter.
- Aliquot into sterile tubes, 5mL samples, label, and freeze for later use.
- The expiration date of the solution is indicated on the Certificate of Analysis and/or the bottle. Document expiration date as date of CoA.
- Diluted solution is good for 1 year frozen (if less than CoA expiration date) and 1 month thawed.

Note

Record media preparation on ***Attachment 1: Solutions Preparation Sheet :***

Attachment 1-Solutions preparation...

- The Cold Chain Complete Card with the WarmMark indicator on the card and the separate ColdMark indicator can both be stored at room temperature or between 12°C to 32°C to ensure against accidental triggering of either indicator.

- Ice Packs and CryoPaks.

Preparation of Ice Packs and CryoPaks prior to isolation:

- **Frozen ice packs:** When creating your -20°C ice packs, place flat in a tub so they freeze flat. At time of packaging, the tub can be easily removed from the freezer.
- **CryoPak 8°C pouches:** When receiving/storing a new box of pouches, allow them to warm up in a hot room or water until the contents liquefy enough to allow the pouches to flatten out. Repackage in tubs or boxes, ensuring that each pouch is flat when storing at 8°C. This again will allow flat 8°C pouches for packaging.
  - *Note: If odd-shaped pouches are the only type available, place them on the sides of the boxes where there is more space, rather than using them on the top or bottom of the shipping container.*

Collection of Islets for Transport

2 After the islets have been allowed to recover from the insult of the isolation process at 37°C for approximately 18-48 hours, the quality of the cultured islets should be assessed by inspecting the islets in several flasks. If the islets have rounded up to greater than 80% of all size islets, the isolation should be ready for shipping. See attachment: Tips for Determining Islet Quality for Shipment.

Attachment 2- Tips for Determining...

2.1 **Preparation of PIM(T) Media:**

- Prepare one 500 mL bottle of PIM(T) media prior to the isolation
- Thaw and add 5 mL of PIM(G)
- Thaw and add 25 mL of AB serum (5% v/v)
- Thaw and add 0.5 mL of prepared ciprofloxacin sterile aliquot
- Once all additives have been added to the bottle of PIM(T), it is now referred to as PIM(T) complete.

Note

If a prepared media bottle is to be used from a previous isolation, it must have been filter sterilized at the end of the previous use. The media will expire within 30 days, once it has been fully supplemented.

Note

Record media preparation on ***Attachment 1: Solutions Preparation Sheet****, of this SOP.*

2.2 Obtain the flasks containing islets from the incubator and examine each culture flask for contamination and aseptically place inside the hood. Obtain one 250 mL conical for each 6 culture flasks and aseptically place inside hood.

2.3 Pool 6 T-175 flasks into each “settling” 250 mL conical by pipetting with a 25 mL pipette wetted with PIM(T). Repeat pooling for each set of 6 flasks into a new “settling” 250 mL conical. Allow conicals to settle while rinsing and checking the flasks.

2.4 Wash the flasks with PIM(T) to collect any remaining islets. Using a 25 mL pipette, deliver 20 mL of media slowly onto the entire bottom surface of the flask in a sweeping motion to loosen islets into the solution. Let the media run to the bottom of the flask by gravity. Repeat this step 2-3 times collecting the 20 mL from the bottom of the flask and delivering it to the top of the flask in a sweeping motion while rocking gently. The same 20 mL can be used for 6 flasks by transferring to the next flask after washing each flask. Collect the wash from all the flasks in to one “count” 250 mL conical.

2.5 Repeat wash on the next 6 flasks and place into the “count conical”.

2.6 After the first wash, examine the flasks under the microscope for stuck islets. If there are many islets attached to the bottom of flask, deliver 20 mL of PIM(T) to the flask for the second time, gently tap on the side of the flask and rock the flask back and forth to get the released islets in to the solution being careful not to get medium into the cap of the flask. Collect the wash and pool in to the “count conical”.

2.7 After the rinses and the “settling conicals” have rested for approximately 10-15 minutes, pre-wet a 25 mL pipette with PIM(T), gently draw the settled islets from the bottom of each of the “settling conicals”, one at a time, by going to the bottom of the conicals containing the settled islets, and pipette with upward movement with only ~ 2-3 mL of media and carefully deliver to the bottom of 250 mL “count conical”.

2.8 Centrifuge the supernatant in the “settling conicals” for 2 minutes @180 g.

2.9 After centrifugation, evacuate supernatant. Gently resuspend the pellets from the “settling conicals” with 2-3 mL of PIM(T) and transfer into the “count conical”.

2.10 Bring count sample up to 200 mL with PIM(T). Take count samples by inverting 250 mL conical 3-4 times, quickly remove cap and have second technician remove exactly 0.5 mL form the “count conical” and place in counting dish. Place the conical containing islets horizontally under the hood while waiting for results from the evaluations.

2.11 According to the islet counts, calculate the concentration of islets/mL and record in the batch record.

Attachment 3-Islet Tabulation Coun...

2.12 Complete the broadcast records and proceed to broadcast. From this value, calculate how much volume is needed to be distributed into each shipping bottle for each order. Record the value in the batch record.

2.13 Remove 100 IEQ for Viability Test and proceed according to IIDP protocol.

2.14 Remove 400 IEQ for GSIR studies and culture per IIDP protocol.

2.15 As investigator's orders are determined through the broadcast system, prepare appropriate amounts of islets into proper sized shipping vessel.

- 30 mL bottle for shipments of less than 10,000 IEQ
- 60 mL bottle for shipments of 10,000-50,000 IEQ
- 125 mL bottle for shipments of greater than 50,000IEQ - 100,000 IEQ

2.16 Distribute appropriate volume into shipping bottles.

2.17 Bring the volume up with PIM(T) stored at 6-10ºC up to the halfway point of the neck of each shipping bottle. ***Note: Specific volumes for specific bottles sizes are as follows:***

- 30 mL bottle – 44 mL of media plus islets
- 260 mL bottle – 75 mL of media plus islets
- 125 mL bottle – 167 mL of media plus islets

2.18 Label each bottle with RRID #, Date, #IEQ, and PI Name

2.19 Record appropriate information on the batch record.

2.20 Keep the shipping bottles with islets in the refrigerator set at 6-10º C until ready for packaging.

Packaging of Islets

3 Stage all materials needed for packaging the islets and ensure all ice packs and CryoPak pouches are at the proper temperature.

Preparation prior to packaging:

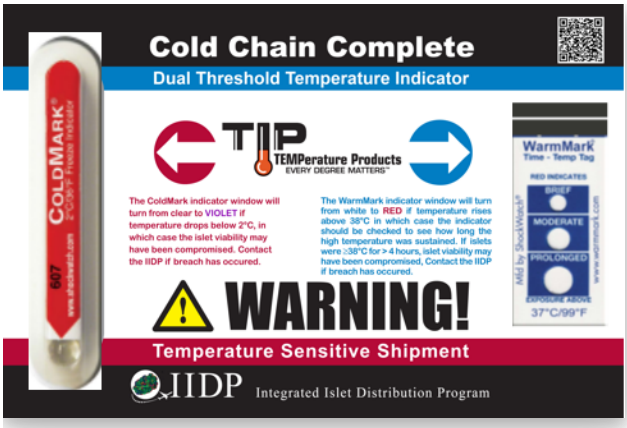
- Determine the number of boxes needed for the shipment and line them up, in advance, including the smaller, inner box
- Prepare your bottles and store in the refrigerator at 8°C.
- Determine the number of 8°C CryoPak pouches, 8°C cold packs, and -20°C frozen ice packs that will be needed, and place all on a cart so that everything is quickly accessible.
- Put the absorbent pads, Ziploc bags, FedEx envelopes, and bottle labels on a second cart.
- Lay out all of your paperwork, so that you can quickly grab it and match it to a bottle.
- Group your bottles by IEQ, to ease the placement in specific packages.
- Follow the protocol and pack the boxes.It should take 2-3 minutes per box.

3.1 The Cold Chain Complete Card is shipped with the WarmMark Indicator attached.

- Store the Card at least 5ºC (9º F) below the **activation temperature of the adhered WarmMark of 37°C** (therefore less than 32º C). Room temperature is acceptable.

- The ColdMark is provided separately because it is always active and should be stored at least 10°C (50ºF) above **activation temperature of ColdMark of 2ºC** (therefore more than 12ºC).Room temperature is acceptable.

- When ready to use, *adhere the ColdMark indicator to the card and activate the WarmMark*. **ACTIVATE** the WarmMark by folding up and pulling out the tab until completely removed.



- Immediately place the Cold Chain Complete Card along with the islet shipping bottles into a Ziploc bag and seal.
- Include attached Cold Chain Complete Form

Attachment 4 -TipTemp Handling In...

3.2 Line the inner cardboard (S-4434) box with absorbent pad. See attached slide presentation for more information.

Attachment 5-Packaging Islets for ...

- Place the bagged shipping bottle(s) containing the islets in the Ziploc bag into the absorbent pad lined, inner cardboard box.
- Surround bottle with six 8ºC cold packs, one on bottom, one on each of the four sides and one on top.

3.3 Mark the direction for upright bottle. Seal the inner box with shipping tape and stand upright.

3.4 Place one 8°C CryoPak pouch and one 8°C cold pack (stored in the refrigerator) in the bottom of the Styrofoam box.

3.5 Place the inner cardboard box containing the islets on top of the pouch and pack, ensuring that the bottle inside is standing upright and centered in the shipping container.

3.6 Place one 8°C CryoPak pouch and one -20°C frozen ice pack on each long side of the inner box. (Packs/pouches are placed in the larger gap between the inner box and the insulated shipping container.)

3.7 Place one 8°C CryoPak pouch and one 8°C cold pack on the top of the inner box**.** ****NOTE**-All packs should be placed in an alternating method, to ensure two of the same packs are not next to each other in the configuration (See **Attachment 5-Packaging Islets for Cold Shipping** for visual details.)*

3.8 Place the Styrofoam lid on top.

3.9 Place Tissue Shipment Form on top of Styrofoam lid.

3.10 Close the flaps of the shipping box and seal with shipping tape. Add Box label to the Box.

Attachment 6-Generic Institution B...

3.11 Attach FedEx label with recipient FedEx number to box and ship **priority overnight.**

Completing the Distribution Records

4 The shipping center will enter the IEQ to be shipped (Confirm Recipient screen) and will enter FedEx tracking numbers (Islet Tracking) into the Islet Allocation System. In addition, the shipping center should process the shipment by using the on-line FedEx process and click the prompt to alert the recipient of the FedEx tracking number. This will help the recipient investigator follow their shipment through the FedEx system and free up time for the centers and the IIDP.

Note: If the investigator has picked up the islets at the distributing center then the center should check "Yes" in the "Direct Pickup" column and "Yes" under "Islets Shipped?" once they are picked up.

4.1 As soon as the center has entered the number of IEQ shipped and indicated that the investigator will be shipped islets, the investigator's account will be set automatically to "ineligible". This will act similar to "on hold" preventing the investigator from receiving additional targeted offers until the User Feedback Form has been completed.

4.2 The investigators will have the FedEx tracking numbers listed in their History of Islet Offers table.

Citations

David W. Scharp, Jayagowri Arulmoli, Kelly Morgan, Hannah Sunshine, Ergeng Hao. Advances in Human Islet Processing: Manufacturing Steps to Achieve Predictable Islet Outcomes from Research Pancreases
10.21926/obm.transplant.1901052

Ricordi C, Gray DWR, Hering BJ, Kaufman DB, Warnek GL, Kneteman N, et al.. Islet isolation assessment in man and large animals.
http://www.ncbi.nlm.nih.gov/pubmed/2075782